Summary of Informal Discussion on Pharmacologically Protected Areas

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Dr. Louis Thomas opened this session by noting that leukemic involvement of the meninges, brain, and spinal cord has been known for nearly 100 years, and that most of the anti-leukemic drugs do not cross the blood-brain barrier in sufficient quantities to eradicate leukemic cells in the brain and meninges. In his studies, the frequency of leukemic involvement of the central nervous system in patients dying of acute leukemia has not changed significantly for the periods 1953–58 and 1961–63. Dr. Thomas pointed out that many of the histologic features of leukemic cell infiltration in the meninges and "non-neural" areas of the mouse inoculated with L1210 leukemia were similar to those of man. "Although direct observation about the route of spread of leukemic cells to the arachnoid can not be made in the human, it was speculated after examination of the human material that direct spread of leukemic cells from dura to the arachnoid may occur in man as it did in the mouse," i.e., by direct migration and growth through the perivascular and perineural tissues of vessels and nerves which bridge the subdural space. Dr. Thomas has observed that while most of the commonly used anti-leukemic drugs failed to eradicate experimental leukemic cells in the brains of mice, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) appeared to be effective in this respect.

Dr. Rall, in his discussion of experimental studies of the blood-brain barrier, emphasized that the pharmacologic asylum (from drug) provided leukemic cells in areas such as the central nervous system (CNS) can be of critical importance. He noted that certain of the nitrosoureas (small molecules, nonionized at body pH, which are highly lipoid-soluble) can "cure" murine meningeal leukemia because they are intrinsically powerful anti-leukemic agents and, further, they possess the physicochemical properties which permit the free and rapid passage from blood stream into brain and cerebrospinal fluid (CSF). Dr. Rall pointed out that "the locus of the blood-brain barrier probably is the tightly packed sheath of glial and neuronal cells which completely surrounds the brain capillary. The blood-CSF barrier is predominantly the choroid plexus, a solid sheet of epithelial secretory cells between the rich choroidal blood supply and the cerebrospinal fluid." None of the barriers is absolute, but a partial barrier is indeed serious for agents with low therapeutic indices. Dr. Rall noted that the characteristics that determine the entry of compounds into the brain and CSF are very similar but that the routes of exit are different. For methotrexate, low CSF:plasma ratios are observed; 6-mercaptopurine at tolerated dosages also fails to enter the CSF to any great extent; preliminary study suggests that hydrocortisone entry into CSF is less than 10% of the plasma concentration; and methylglyoxal-bis-guanylhydrazone appears to enter the brain and CSF only to a very limited extent. Dr. Rall mentioned the possibility of other areas of pharmacologic asylum such as the thymus or the testes, and the possibly very important "slow entry" of certain drugs into into the center of large tumors because of inadequate blood supply in the interior of the tumor.

Dr. Handschumacher asked Dr. Rall if the effect of stilbanicide on the outflow to the brain of intrathecally administered methotrexate had been studied. Dr. Rall felt that based on other studies it would be difficult to obtain high enough concentrations in this manner.

Dr. Bergel pointed out, in connection with Dr. Rall's comment on the accessibility of the inner portion of solid tumors to drug, that Dr. Goldinger had administered glycerine green and found the inner area of tumors to be inaccessible. Dr. Bergel also referred to work at Bethesda in which differences in tumor penetration by D- and L-tyrosine had been observed. He suggested the possibility of controlling entry of certain agents into the CNS based on optical specificity.

Dr. Day briefly discussed the preliminary experiments of the group at Duke which were concerned with whether radiolabeled antibody can penetrate the blood-brain barrier.

Dr. Bertino asked if Dr. Rall had carried out experiments with folic acid similar to those he described with methotrexate. He felt that it would be of interest to determine if the same transport system applies. Dr. Bertino also commented that 1 very important factor in the possible chemotherapeutic cure of choriocarcinoma in women may be that it is a highly vascular tumor with no drug-protected core.

Dr. Rall answered that the folic acid active transport experiment had not been carried out and that the observations on penetration of agents into the core of tumors were not his, but were published reports.

Dr. Goldin showed a slide summarizing work by Drs. Chirigos and Thomas which he felt bears on the possibility of being able to use immunity in relation to chemotherapy. Mice were immunized or partially immunized against leukemia L1210 and leukemia cells were then inoculated intracranially; the control animals had a median survival time of 10 days; nonimmunized mice treated with bromochloroamethopterin had a median survival time of 38 days (considerably less than ordinarily obtained with the sub-

cutaneously inoculated disease). Mice that were preimmunized and inoculated intracerebrally lived somewhat longer than nonimmunized controls, but mice that were immunized and treated with bromo-chloroaminopterin showed a very long survival time; in fact, there were 7 survivors. Dr. Goldin stated that there is a possibility that we may obtain the kind of selectivity we are looking for in chemotherapy with the aid of some immune mechanism.

Dr. Murphy asked Dr. Thomas if he had any specific information about the local lesion in the rare leukemic patient who appears to have hypothalamic involvement and problems of "eating" syndrome and weight gain.

Dr. Thomas replied, "Yes, in fact the first observation in our own series of cases of diffuse leukemic cell infiltration in the area of the tuber cinereum and around the floor of the 3rd ventricle was in a patient who had a hypothalamic syndrome, ate excessively, and had polydipsia and polyuria." He noted that a number of such cases had been reported and suspected that any case of leukemia which mimics Froelich's syndrome will have leukemic cell infiltration in this region.

Dr. Thomas also commented on Dr. Goldin's remarks. In the model which he and Dr. Chirigos have been using, if there is a "take" at all in the intracerebrally inoculated, immunized animals, leukemic cells grow along the neural tract and form a nodule or a solid tumor. Drs. Chirigos and Thomas have been trying to explore some of the implications and avenues which might explain this.

Dr. Kensler reported that his group has carried out experiments with intracranially implanted Dunning leukemia, leukemia L1798, and leukemia L1210. With 10^6 ascites leukemia cells, diffuse invasion of the brain was observed. Dr. Kensler stated that even under these conditions Southern Research Institute's compound, BCNU, still was effective as a chemotherapeutic agent, although cytoxan was not.

Dr. Skipper remarked that it is perfectly all right to refer to BCNU as Southern Research Institute's compound when one is talking about its capacity to cross the blood-brain barrier and kill leukemic cells in the CNS; however, when the subject of its delayed host toxicity is under discussion, he would prefer that the compound be referred to as the Cancer Chemotherapy National Service Center's (CCNSC's) compound.

Dr. Skipper pointed out that contributions to the development of BCNU came from numerous sources: (*a*) the activity of 1-methyl-1-nitroso-3-nitroguanidine was discovered in the CCNSC screening program; (*b*) Dr. John Montgomery suggested evaluation of 1-methyl-1-nitrosourea, which proved to be more effective against leukemia L1210; (*c*) Dr. Frank Schabel discovered the activity of 1-methyl-1-nitrosourea against intracerebrally inoculated leukemias; (*d*) Dr. B. R. Baker (then at Stanford Research Institute) and associates prepared 1-(2-chloroethyl)-1-nitrosourea, which is more active than 1-methyl-1-nitrosourea; and (*e*) Dr. Tom Johnston, Dr. Montgomery, and their associates prepared BCNU which is somewhat more effective than the monochloroethylnitrosourea against intracerebrally inoculated L1210 leukemia. Thus, the ownership of BCNU is as diagrammed in Chart 1.

Dr. Farber remarked as follows: "I have 2 comments. First, I want to express my appreciation for these 2 superb papers, and secondly, it does one's heart good to see such fine photomicrography. I am very much interested in the size of the infiltrates that you have seen, Dr. Thomas, and that others have encountered too. This would not be expected from clinical experience, and I wonder if Dr. Lois Murphy, Dr. Sutow, Dr. Mila Pierce, and Dr. Burchenal might say a word about the extreme rapidity of disappearance of symptoms of central nervous system involvement and clearing of the spinal fluid after 1 intrathecal injection of methotrexate, and I wonder how Dr. Thomas feels about such clinical observations in view of his findings at autopsy."

Dr. Thomas mentioned that he wanted to be certain that

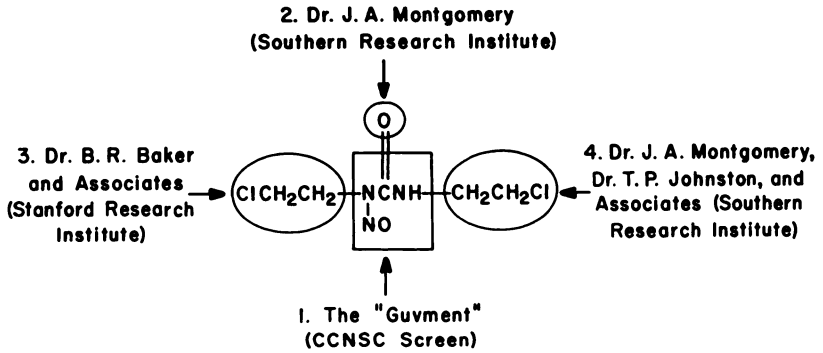

CHART 1.—Stepwise drug design contributions which, when coupled with biologic activity data (intracerebrally inoculated L1210 leukemia system), led to BCNU, which crosses the blood-brain barrier. *1*, 1-Methyl-1-nitroso-3-nitroguanidine, uncovered in the CCNSC screen, very weakly active against intraperitoneally inoculated L1210 leukemia and not active against intracerebrally inoculated disease. *2*, 1-Methyl-1-nitrosourea, suggested by Dr. Montgomery, moderately active against both intraperitoneally and intracerebrally induced L1210 leukemia. *3*, 1-(2-Chloroethyl)-1-nitrosourea, prepared by Dr. Baker and associates, more active against both intraperitoneally and intracerebrally induced L1210 leukemia. *4*, 1, 3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) prepared by Dr. Johnston, Dr. Montgomery, and associates, more active against intracerebrally inoculated L1210 leukemia.

there was no misunderstanding about what he had presented. When one looks at a considerable quantity of end results over a long period, they are somewhat discouraging to those who are trying to eradicate leukemic cells from the body. However, Dr. Thomas stated that "there is no question but what some systemic therapy, certainly with the steroids, and some intrathecal therapy seem to wipe out most of the leukemic cells." Dr. Thomas also pointed out that leukemic cell infiltration of peripheral nerves has been studied by others and that these infiltrates produced symptoms which cleared very rapidly with systemic therapy.

Dr. Freireich commented on the tremendously effective temporary clinical control of meningeal leukemia with intrathecal therapy. Dr. Freireich and his associates have looked back at the clinical course of patients on which Dr. Thomas' study was based. Dr. Freireich stated "it is the partially treated patients, particularly the ones who have had remissions, that have opportunity to develop meningeal disease. At least in a qualitative way one can correlate the frequency of meningeal infiltration, the duration of life, and the effectiveness of treatment. In the early series, we found that 40% of the patients had meningeal disease at autopsy, but we had recognized clinically only about 16% of patients as having the clinical symptoms so it was clear that extensive arachnoidal infiltration could exist in the absence of symptoms." Since then Dr. Freireich and his associates have examined spinal fluid for the presence of leukemic cells and found that 65% of patients with lymphoblastic leukemia had gliocytosis with elevated cerebrospinal pressure. Of these patients who had meningeal leukemia, 9 (which Dr. Freireich considered encouraging) could not be shown to have meningeal leukemic disease at time of autopsy (about half of these received steroids in the interim and the other half had received only intrathecal aminopterin). On the other hand, for the majority of patients who have meningeal leukemia at any time, recurrence is the rule rather than the exception. Of the patients whom Dr. Thomas found at autopsy to have this disease, the overwhelming majority were predicted by the cerebrospinal findings before death.

Dr. Farber remarked that when you find no evidence of leukemia in the brain or meninges at autopsy you speak only of those sections that you examined under the microscope and that there are miles and miles of space which you have not examined at postmortem. Thus, he felt that use of the word "cure" in this dimension might be misleading to some less sophisticated than those in attendance.

Dr. Murphy showed a slide which summarized the course of a 14-year-old girl with leukemia. The cerebrospinal cell count was 1700 cells when she received a first intrathecal dose of methotrexate (0.25 mg/kg); her symptoms improved but the count increased to 3300. Treatment was then continued every 2nd or 3rd day, the cerebrospinal count returned to normal (as did the CSF sugar level) and her subsequent course was free of any of the manifestations of the disease. She died about 8 months later of other manifestations of leukemia.

Dr. Zuelzer commented that the frequency with which the clinical manifestations of meningeal leukemia can be controlled has, in his experience, been high. Very few patients in a series of 51 that have been recognized have died as a result of the CNS disease; they have died eventually of bone marrow disease or generalized leukemia. Dr. Zuelzer feels that the ophthalmoscope now belongs as much in the regular routine examination of CNS disease as do the microscope and the manometer. Dr. Zuelzer remarked that in their series, under the therapeutic conditions employed, the incidence of *de novo* CNS involvement was rather steady throughout the 1st 3 years, decreasing, of course, in absolute numbers because the number of patients in the group keeps decreasing. "The percentage was rather constant in each period, something approaching 20%, but at 36 months, this did become discontinuous in the sense that Dr. Burchenal defined it; any survivor that has not, in our series, developed CNS involvement by 36 months will not develop it, no matter how long they live and no matter whether they subsequently have and die of hematologic relapse. This point might be worthy of serious thought."

Dr. Kaplan remarked that it is possible to irradiate the entire cerebrospinal axis effectively in various clinical situations, "in terms of Dr. Skipper's exponential cell kill model, there may be a situation in which intrathecal methotrexate kills down to a very few logs of cells where the finishing touches might well be achieved by a perfectly tolerable dose of radiotherapy." Dr. Kaplan asked if anyone had clinical or pathologic information on this approach.

Dr. Levin stated that such a study was now in progress at the National Cancer Institute. The approach is to employ systemic therapy, aminopterin intrathecally (to get the leukemic cell number down to as low a level as possible), and then irradiate the brain and spinal cord. The present schedule is 1800 r in 6 days.